Neurophysiological markers associated with heterogeneity in conduct problems, callous unemotional traits and anxiety

Running Head: HETEROGENEITY AND NEUROPHYSIOLOGY

Neurophysiological markers associated with heterogeneity in Conduct Problems, Callous Unemotional Traits and Anxiety: Comparing children to young adults

Abstract

Evidence from physiological studies has been integral in many causal theories of behavioral and emotional problems. However, this evidence is hampered by the heterogeneity characterizing these problems. The current study adds to prior work by identifying neuro-physiological markers associated with heterogeneity in conduct problems (CP), callous-unemotional (CU) traits and anxiety. Participants were classified into the following groups: a) low risk, b) anxious (predominately high anxiety), c) primary (scored high on CP and CU traits but low on anxiety) and d) secondary (high anxiety, CU traits and CP). Developmental differences were also examined by including two different samples assessed during young adulthood (Study 1: *N*=88; *M*age=19.92; 50% female) and childhood (Study 2: N=72; *M*age=5.78, *SD*=1.33; 39 males). Participants in both studies were recruited from community samples (Study 1: *N*=2306; *M*age=16, *SD*=.89; Study 2: *N*=850; *M*age=5.01, *SD*= .95). Physiological responses (heart rate, skin conductance, startle modulation) were recorded while children and adults watched negative affective and neutral scenes. Medial prefrontal activation (Oxygenated Hemoglobin) was also measured in young adults. Findings suggested that individuals in the secondary and anxious psychopathy groups showed higher physiological arousal and startle reactivity to violent, fearful, and anger stimuli compared to individuals in the primary psychopathy group. In contrast, primary and secondary psychopathy groups showed similar physiological reactions to sad stimuli assessed during childhood. Also, young adults in the primary and secondary subtypes showed lower medial prefrontal cortex activation to violent stimuli compared to the anxious group. These findings provide evidence for the value of a multi-domain approach for clarifying neurophysiological mechanisms that can inform prevention and treatment efforts.

Keywords: Conduct Problems; Callous-Unemotional; Anxiety; startle; heart rate and skin conductance; medial prefrontal cortex.

Introduction

Antisocial behavior is defined as non-age appropriate, persistent, and repetitive disruptive acts (e.g. bullying, vandalism) that violate the rights of others or social norms (Frick & Morris, 2004). As such, antisocial behaviors are associated with a host of individual impairments (e.g., social, emotional, academic) as well as public and economic burden to society (Fanti & Henrich, 2010; Huesmann, Dubow, & Boxer, 2009; Moffitt, 1993; National Collaborating Centre for Mental Health, 2013; Scott, Knapp, Henderson, & Maughan, 2001). The serious difficulties among antisocial youth exhibiting severe conduct problems (CP: e.g. fighting, assaulting, lying and stealing) highlighted the need for a more comprehensive understanding of these behaviors that led to several causal and development theories (e.g., Fanti, 20016; Raine, 1993). Recent empirical and clinical research indicated that the most notable aspect of these problems is that there is remarkable heterogeneity, with some individuals showing co-occurring emotional problems (Fanti & Henrich, 2010; Schoorl, Van Rijn, De Wied, Van Goozen, & Swaab, 2016) and others characterized by psychopathic personality traits (Fanti, 2016; Frick et al., 2013).

Further, despite advances in neuroscience, psychology, and psychiatry, there is no agreement in terms of neuro-physiological markers associated with heterogeneity in antisocial beaviors (e.g., Beauchaine, 2012; Fanti, 2016). In a review of the literature, Fanti (2016) provided evidence that taking into account callous-unemotional (CU) traits (i.e., lack of remorse/empathy, callous use of others, shallow/deficient affect) in addition to anxiety can result in meaningful classifications for CP at both the phenotypic and physiological level. According to this review, unique CP subgroups score on opposite extremes on multiple neuro-physiological measures. Individuals high on both CP and CU traits are characterized with physiological hypo-arousal, fearlessness and normative emotion regulation, whereas individuals high on CP and anxiety are characterized by hyper-arousal, fearfulness and

emotional dysregulation. Failing to take these individual differences into account might have contributed to the inconsistent findings reported in prior neuro-physiological studies.

Additionally, a number of studies with adolescents and adults suggest that antisocial individuals scoring high on CU traits can be distinguished into those with increased levels of anxiety (i.e., secondary psychopath), and those with low or average anxiety levels (i.e., primary psychopathy) (e.g., Drislane et al., 2014; Fanti, Demetriou, & Kimonis, 2013; Kimonis, Skeem, Cauffman, Dmitrieva, 2011). A recent article provided evidence that high and low levels of anxiety can actually differentiate secondary from primary psychopathy groups as early as age 3, and these subtypes identified early in life demonstrated developmental stability across a period of 12 years into adolescence (Fanti & Kimonis, 2017). These findings provide support for the utility of anxiety for differentiating primary and secondary psychopathy groups across development. This approach is rooted in Karpman's developmental theory (1941, 1948a, b), who suggested that traumatic environmental experiences, parental abuse, and rejection is associated with the development of psychopathic traits co-occurring with anxiety, forming the secondary psychopathy group (Mealey, 1995; Porter, 1996). Indeed, high levels of CU traits and anxiety have been associated with histories of abuse and maltreatment (Dadds, Kimonis, Schollar-Root, Moul, & Hawes, 2017; Kahn et al., 2013; Kimonis, et al., 2011). Theoretically, the affective deficits found among individuals in the secondary psychopathy group, related to anxiety and CU traits, develops as an adaptation mechanism to traumatic environmental experiences (Fanti & Kimonis, 2017; Karpman, 1941, 1948a, b; Porter, 1996).

In contrast, primary psychopathy develops due to an individual's inherent personality and temperamental deficits that lead to deficient empathic concern (Hicks, Markon, Patrick, Krueger, & Newman, 2004). The lack of conscience predisposes this antisocial subgroup to be less responsive to negative affective information and less sensitive to cues of distress (i.e.,

low anxiety) and punishment (Fanti, Colins, Andershed, & Sikki, 2016b; Frick, Ray, Thornton & Kahn, 2014). Theoretically, the weak behavioral inhibition, fearless temperament and low reactivity to stress shown by individuals in the primary group prevents them from engaging in important socializing cues, resulting in severe and violent antisocial behavior with low remorse or guilt (Cleckley, 1941; Fanti et al., 2013; Fanti & Kimonis, 2017).

Contemporary research on psychopathy subgroups predominately distinguishes individuals in the primary group as more "emotionally unresponsive" than those in the secondary group, based on their reduced reactivity and stress reaction. On the other hand, the secondary group is characterized as "aggressive" with high negative emotionality, increased level of impulsivity and irresponsibility, leading to more dysregulated emotional behaviors (Hare, 2003; Hicks et al., 2004; Hicks & Patrick, 2006; Lykken, 1995; Verona, Patrick, & Joiner, 2001). As a result, the secondary psychopathy group might resemble anxious individuals in terms of their emotional difficulties and over-reactions to affective stimuli, indicating that anxiety might drive differences in emotional reactivity (see Fanti, 2016 for a review).

The current study aims to take individuals differences into account, and investigate how low risk (i.e., normative scores on these measures), predominately anxious, primary, and secondary psychopathy groups differ on distinct neuro-physiological markers associated with arousal (heart rate and skin conductance), valence (startle reflex), and medial prefrontal cortex functioning. Integrating information from individual and neuro-physiological measures can advance both basic and applied research, leading to empirical and practical implications. A novel aim of the present study is the investigation of whether physiological responses are similar or different among children compared to adults, advancing prior work which is limited to adolescent and young adult populations.

Responsiveness to emotional stimuli

The vast majority of previous studies found that impairments in the recognition and responsiveness to adverse stimuli are a prominent feature found among individuals with CU traits (Blair, 2013; Fanti et al., 2016 a, b, c). More specifically, both adults and children with elevated CU-traits show deficits in recognizing fearful and sad expressions, as well as reduced responsiveness to others' cues of distress and fear (Blair, 1999; Blair et al., 2004; Fanti et al., 2016 a, b, c; Frick & White 2008; Kimonis et al., 2006). Thus, abnormal processing of emotions may underlie the absence of empathy and guilt among these individuals. Despite the fact that deficits in emotional processing towards negative emotions have been supported consistently across a number of studies, there are several inconsistent findings among them. For instance, Woodworth and Waschbusch (2008) found that children high on CU-traits recognized sadness less accurately, but they were more accurate on fear identification. Others suggested that CU-traits are associated with impairments in identifying fear, but not sadness (Leist & Dadds, 2009). One possible explanation for these mixed findings can be attributed to the heterogeneity of this group, which needs to be taken into account. The presence or not of anxiety may add to these contradicting evidence on the basis of how the two psychopathy sub-groups respond to negative emotional stimuli.

Currently, there is only limited work examining differences in affective responses between primary and secondary groups (e.g., Fanti & Kimonis, 2017; Kimonis, Cauffman, Goldweber, & Skeem 2012). Existing work suggests that individuals in the primary group demonstrate decreased facilitation to fear and sadness cues, due to their failure to attend to signs of distress in others (Kimonis, Cauffman, Goldweber, & Skeem, 2012). In contrast, anxious youth, irrespective of CP, exhibit a hyper-sensitivity towards anger and fearful stimuli (Fanti, 2016; Masten et al., 2008). Despite the low empathy and high CU traits that characterize the secondary psychopathy group, individuals in this group show similar emotional perception deficits as those high on anxiety, and experience negative affect and

high arousal in emotionally charged situations (e.g., Bagley, Abramowitz, & Kosson, 2009; Fanti, 2016; Williamson, Harpur, & Hare, 1991; Richell et al., 2005). However, no prior work compared these groups in terms of their physiological reactions to emotional stimuli. By doing so, we aim to test whether the physiological reactions of these subtypes are driven by their levels of anxiety or CU traits.

Distinct associations with neuro-physiological measures

Among normative populations, emotions serve an adaptive role and are important for future survival and well-being (Posner, Russell, & Peterson, 2005; Lang, Greenwald, Bradley, & Hamm, 1993). Multiple systems are involved in the generation and the interpretation of feelings of differential valence (pleasant-unpleasant continuum) or arousal (low-high activity/alertness), and there are differences in the degree to which an individual can cope (i.e., anxiety) or react (e.g., low empathetic behavior) to different emotions (Fanti, 2016). Affective reactions to emotional stimuli influence social interactions, and we expect that dysfunctions in the systems that regulate emotions can also explain individual differences in antisocial behavior, CU traits and anxiety. Despite this evidence, the majority of prior work focuses on one biological system at a time, and the current study aims to compare heterogeneous groups on multiple neuro-physiological systems. Physiological measures are less biased as opposed to self- or parent-reports, and can provide information regarding brain processes related to maladaptive behaviors or emotions. In the current study we focus on: Heart Rate, Skin Conductance, and the eye-blink startle reflex assessed during childhood and adulthood. We also expect medial prefrontal cortex activity assessed during adulthood to be associated with abnormalities that predispose to CU traits, anxiety, and CP, indicating that it is important to understand dysfunctions in both cortical and physiological systems.

Heart Rate (HR) and **Skin Conductance (SC)** are the most popular physiological measures of general emotional arousal, and multiple theories of antisocial behavior and

anxiety are based on these measures (Fanti, 2016; Lorber, 2004). SC reflects primarily Sympathetic Nervous System (SNS) activity, while HR reflects both Parasympathetic Nervous System and SNS activity. When assessed at rest, HR and SC reflect autonomic activity in the absence of external stimuli, and reactivity is expressed as a change from resting or as a difference score from neutral after exposure to affective stimuli (e.g., emotional evoking film clips) (Fanti, 2016; Fanti et al., 2017a). Hyper-arousal (i.e., high SC and HR) is associated with oversensitivity in fearful or threatening situations and problems in emotion regulation, whereas hypo-arousal (i.e., low SC and HR) is associated with thrill seeking and risky behaviors that can raise physiological arousal and excitement (Beauchaine, 2012; Frick & Morris, 2004). In addition, hypo-arousal is associated with reduced reactivity to fearful or threatening situations, which inhibits moral and conscience development and increases the likelihood of engaging in antisocial acts (Fanti, 2016; Raine, 1993).

Anxious individuals are characterized by hyper-arousal and research has revealed that individuals high on anxiety with or without co-occurring CP show high physiological reactivity to emotional situations (Fanti, 2016; Mezzacappa et al., 1997, Rogeness, Cepeda, Macedo, Fisher, & Harris, 1990; Schoorl et al., 2016). In contrast, children, adolescents and adults high on CU traits show physiological under-arousal and display low HR and SC reactivity to aversive stimuli (Anastassiou-Hadjicharalambous & Warden, 2008; Blair, Jones, Clark, & Smith, 1997; de Wied, van Boxtel, Matthys, & Meeus, 2012; Northover, Thapar, Langley, & van Goozen, 2015; Kimonis, Frick, Munoz, & Aucoin, 2008; Patrick, 1994). These results are consistent with theory and studies proposing that CU traits are associated with fearlessness (e.g., Fanti, Panayiotou, Lazarou, Michael, & Georgiou, 2016b; Lykken, 1995). However, not all studies find significant or expected associations between anxiety and CU traits with SC and HR measures (e.g., Fanti et al., 2017a; Hoehn-Saric,McLeod, Funderburk, & Kowalski, 2004; Lorber, 2004; Rosebrock, Hoxha, Norris, Cacioppo, &

Gollan, 2016), indicating that it is important to account for heterogeneity between these measures. When taking heterogeneity into account during childhood, Fanti and Kimonis (2017) indicated that the primary group scored lower than the anxious and secondary groups on resting heart rate. Unfortunately, it is not clear from prior work whether primary and secondary psychopathy group can be distinguished based on their SC and HR reactivity to emotional stimuli, since the majority of studies assessing physiological reactions among individuals high on CP and CU traits do not differentiate them into primary and secondary psychopathy groups. In addition, no prior work compared anxious individuals with or without CU traits on HR and SC measures assessed during emotional stimuli.

The **eye-blink startle reflex** is an involuntary response to a sudden intense acoustic stimulus and is a well-established measure of defensive motivation that is modulated by dimensions of affect (Fanti, Panayiotou, Kyranides, & Avraamides, 2016a; Kramer, Patrick, Krueger, & Gasperi, 2012; Patrick, 1994; Vaidyanathan, Patrick, & Cuthbert, 2009; Vrana, Spence, & Lang, 1988). The reflex's amplitude is typically potentiated by negatively-valenced affective contexts (i.e., fear, threat, victim scenes, and assault) in relation to neutral situations, and is attenuated during the presentation of positive stimuli. Reduced startle potentiation to negative and threatening stimuli reflects diminished amygdala activity (Fanti, 2016). Anxious individuals exhibit potentiated startle in response to threatening stimuli (Kaviani et al., 2004; Miller & Patrick, 2000; for a review see Fanti, 2016 and Grillon & Baas, 2003), whereas antisocial children and adults high on CU traits are characterized by reduced eye-blink startle potentiation during exposure to aversive and fearful emotional stimuli (Dackis, Rogosch, & Cicchetti, 2015; Fanti et al., 2016a, b; Kyranides, Fanti, & Panayiotou, 2016). Limited evidence also points to a continuum of low to high startle modulation in response to fearful and threatening stimuli among primary and secondary psychopathy groups. Similar to those high in anxiety, individuals in the secondary

psychopathy group show high startle potentiation to threatening stimuli, although those in the primary psychopathy group show startle attenuation to this stimuli (Dackis et al., 2015; Kimonis, Fanti, Goulter, & Hall, 2016). Moreover, no differences in emotional stimuli of positive valence (i.e., erotic, comedy) were identified in previous studies, suggesting that aversive and threatening stimuli have a greater biological relevance for antisocial behavior and anxiety (Fanti et al., 2016b; Kyranides et al., 2016). However, the majority of studies focused on adolescents and adults with CU traits or anxiety, with very few studies attempting to extend startle modulation findings to children (see Fanti et al., 2016 for an exemption). Importantly, no prior work compared anxious individuals to primary and secondary psychopathy groups on startle modulation.

In addition to physiological responses, in order to interpret and contextualize emotional information requires investigation of brain systems involved in generating emotional experience. The **medial prefrontal cortex** (**mPFC**) has a general role in emotion processing and is consistently activated across emotions of positive or negative valence (Fanti et al., 2016c; Phan, Wager, Tyalor, & Liberzon, 2002). It has been established that mPFC evaluates emotional stimuli in terms of future survival and well-being, and by doing so it generates feelings and influences decision making (Etkin, Egner, & Kalisch, 2011; Posner et al., 2009). Individuals high on CU traits show weaker mPFC engagement in response to empathy-eliciting and threatening stimuli, while anxious individuals show stronger mPFC engagement in these situations (Frick et al., 2014; Posner et al., 2009). Prior work using functional near infrared resonance (fNIRS) imaging agree with these findings, providing support of the fNIRS methodology utilized in the current study (Fanti et al., 2016c). An interesting finding is that similar to those high in empathy, anxious individuals show exaggerated mPFC activity during empathy eliciting tasks (Balconi, Bortolotti, & Gonzaga, 2011; Etkin et al., 2011; Wagner et al., 2009), and it is unclear how anxious individuals low

on empathy (secondary) will react to stimuli displaying others in distress. No prior work compared primary, secondary, and anxious groups on mPFC activity.

Current study

Even though there is a growing body of research investigating the general emotional processing deficits of antisocial individuals using physiological measurements (Fanti, 2016), no prior work compared heterogeneous groups of individuals differentiated on anxiety, CP, and CU traits in terms of their physiological reactions to emotional evocating stimuli. The current study aims to examine whether low risk, anxious, primary and secondary psychopathy groups show distinct neuro-physiological responses to aversive stimuli during childhood and adulthood. We propose that it is essential to consider simultaneously multiple physiological responses to emotional stimuli to understand individual differences in emotional experience.

In study 1, we assessed HR and SC at rest, and neuro-physiological (i.e., HR, SC, startle modulation and mPFC activity) responses to violent and neutral video stimuli in young adults classified in primary, secondary, anxious and low risk groups during adolescence. In study 2, children classified in the same four groups were followed one year later and were compared on physiological measures of HR, SC, and startle modulation, assessed while children viewed cartoon scenes eliciting sadness, fear, and angry emotions. Neutral and low arousing scenes were used as a control condition in both studies. Several tasks have been used to elicit different emotional states in previous studies, such as pictures (Kimonis et al., 2006), imagery (Fanti et al., 2016b) and movie scenes (de Wield, van Boxtel, Matthys, & Meeus, 2012; Kyranides,Fanti, & Panayiotou, 2015). In the current study, we decided to use movie scenes instead of static pictures, as movie scenes are more ecologically valid and realistic and include both optical and auditory stimuli. Moreover, current work advances prior investigations by using a multi-method assessment of different physiological systems to compare the identified groups. Further, by testing these associations in both children and

adults we will be able to examine developmental similarities in physiological responses, an important endeavour especially since limited physiological research focuses on children.

Based on previous findings, individuals in the primary psychopathy group (high on CP and CU but low in anxiety) are expected to be characterized by low arousal at rest (i.e., low HR and SC), physiological hypo-arousal as indicated by HR and SC reactivity, low eye-blink startle potentiation, and low mPFC activity in response to negative stimuli. These findings follow suggestions that individuals in this group show low emotional responsivity to aversive stimuli. On the contrary, due to their affective deficits, lower emotional regulation and higher sensitivity to threatening cues, individuals high on anxiety, irrespective of CU traits, may be more likely to experience hyper-arousal, high startle potentiation, and high mPFC activity in response to emotionally charged situations (Fanti, 2016). As a result, it is hypothesized that anxiety might drive the physiological responses of both anxious and secondary groups. Developmental differences and distinct responses to multiple emotions will also be tested. Testing these associations across different developmental stages is important because chronological age was found to explain differences in physiological reactivity during exposure to emotional stimuli (e.g., Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). In addition, since the secondary psychopathy group processes differently fear and sadness information compared to the primary group, it is critical to investigate emotional processing in response to specific emotions (sad, fear; see Kosson, Suchy, Mayer, & Libby, 2002), which we will take into account in the child study.

Study 1 Method

Participants and screening

Study 1 was conducted to compare young adults that were classified in normative (i.e., low risk), primary, secondary or anxious profiles during adolescence on multiple neuro-physiological measures (HR, SC, startle reflex, mPFC). Data were collected during two

developmental stages: adolescence and young adulthood. At initial assessment, 12 high schools in three Greek-Cypriot provinces (Nicosia, Larnaca, and Limassol) were randomly selected for participation, following approval of the study by the Cyprus Ministry of Education and Culture and Cyprus Bioethics committee. Parents/ guardians were informed of the longitudinal nature of the study and 91% of those contacted consented to their child's participation in the study. Ninety five percent of students assented to participate. The final sample consisted of 2306 (*M* age = 16, *SD* = .89) high school students, and was divided evenly between boys (*n* = 1160) and girls (*n* = 1146).

Adolescents differing on levels of CU traits, CP and anxiety were selected based on a Latent Profile Analysis conducted in prior work (Fanti et al., 2013). As reported by Fanti and colleagues (2013), a model with four-classes (BIC = 41,354; AIC = 41,251; LMR < .05) fit the data better than the three (BIC = 41,490; AIC = 41,410; LMR < .05) and five-class (BIC = 41,837; AIC = 41,963; LMR = .35) models based on the Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the Lo-Mendel-Rubin (LMR) model fit statistics, with mean posterior probabilities ranging from .88 to .98 and the entropy value being .91 (see Figure 1): The low risk group scored below average on anxiety, CP and CU traits, the anxious group scored high on anxiety but low on CP and CU traits, the primary group scored high on CU traits and CP but low on anxiety, and the secondary group scored high on anxiety, CU traits, and CP (for additional information please refer to Fanti et al., 2013). From the groups identified during adolescence, 101 participants (*M*age = 19.94, *SD* = .97) were randomly identified and were contacted approximately four years later to participate in the study. From those invited, 88 (*M* age = 19.91, *SD* = 1.01; 50% female) completed the experimental session, in which their responses (physiological and subjective) to affective and neutral video stimuli were assessed. These participants formed the four groups of interest (primary: n=23, secondary: n=23, anxious: n=20; low risk: n=22). All

participants provided consent for participating in the experimental phase of the study and received a small monetary reimbursement (€15).

Questionnaires *(All questionnaires were administered in Greek, during adolescence and adulthood)*

Callous-unemotional traits. The Inventory of Callous-Unemotional Traits (ICU; Frick, 2004) is a 24-item scale designed to assess self-reported callous-unemotional traits. The ICU comprises 12 positively worded (e.g., "I express my feelings openly") and 12 negatively worded items (e.g., "What I think is "right" and "wrong" is different from what other people think") that are rated on a 4-point Likert-scale ranging from 0 (not at all true) to 3 (definitely true). Item scores are summed to form a total score that demonstrated adequate internal consistency in the present study during adolescence (α = .80) and adulthood (α = .85). Previous research has provided evidence for the validity of ICU scores in community and high risk samples of adolescents and young adults in USA and Cyprus (Fanti et al., 2013, 2017; Fanti, Frick & Georgiou, 2008; Kimonis et al., 2008). CU traits (r = .78, p < .001) were stable across the four years under study.

CP and anxiety. The Checkmate plus Youth and Adult Inventories (Gadow & Sprafkin, 1999) are self-report checklists for the most common mental disorders. Participants rate symptoms on a 4-point Likert scale ranging from 0 (never) to 3 (very often). For the purposes of the present study, only items corresponding to the 15-item Conduct Disorder (e.g., "I have stolen things from others using physical force"; adolescence α = .90/ adulthood α = .92) and 6-item Anxiety (e.g., "I have trouble getting myself to stop worrying"; adolescence α = .85/ adulthood α = .86) scales were used. Items were summed to create overall CP and anxiety scales. Previous research has provided evidence for the validity of these instruments in community and clinical samples in Cyprus and USA (Fanti et al., 2013; Gadow & Sprafkin, 1999; Kyranides et al., 2016). Time 1 and time 2 anxiety (r = .69, p <

.001) and CP (r = .65, p < .001) were highly correlated over time.

Experimental procedure

Upon arrival at the lab, participants were briefed about the procedure and provided consent. Participants were then seated in a padded reclining chair, fitted with the physiological sensors and fNIRS headband in a room with dim lighting. Participants were instructed to relax in order to check the effectiveness of recordings. Baseline physiological activity was recorded for a 60-s period while participants viewed a blank screen. Next, a fixation point appeared in the center of the screen for 5 s, followed by the video scene. Participants viewed a total of 12 video scenes, which were presented in a randomized order. After the experiment, physiological sensors were removed and participants were debriefed.

Experimental Materials

Six violent and six neutral scenes, each of 1-min duration, were used in the current study (for validation and norming information see. Violent scenes were selected to differ on valence (pleasantness) and to be higher on arousal than neutral scenes. Violent scenes were excerpts from cinematic productions and included video segments from the following feature films: *Law Abiding Citizen (2009)*, *American History X (1998)*, *Lucky Number Slevin (2006)*, *Rambo 4 (2008)*, *The Killer Inside Me (2010),* and *The Brave One (2007)*. All scenes included realistic depictions of shooting, fighting, beatings, amputations, stabbings, and contained violence for the entire 60-s duration. Neutral scenes depicted nature scenes with little human or animal activity (e.g., Himalayan, Solar System, Andes, Limestone, Tanami desert, Hoodoos). Scenes included music and some dialog or commentary of approximately equal duration across categories. All scenes were in English and video soundtracks were reduced in volume such that the mean volume across each scene was 70-dB, in order to ensure that the acoustic startle probes (see below) could be easily perceived.

Apparatus. The timing of events, the presentation of auditory and visual stimuli, and the

recording of participants' responses were controlled by an E-Prime 2.0 script (Schneider, Eschman & Zuccolotto, 2002). Auditory stimuli (i.e., video soundtrack and startle probes) were presented binaurally via supra-aural headphones in order to mask ambient noise. Visual stimuli were presented on a computer screen (47cm x 24.5cm) placed 150 cm from the participant. All physiological signals were collected using BIOPAC MP150 for Windows bioamplifiers and transducers, running Acq4.2, COBI studio data acquisition and fNIRSOFT analysis software (Biopac Systems Inc, Santa Barbara, CA). Physiological measures were continuously monitored during the recording session and visually inspected offline.

Startle probes. Noise probe stimuli for eliciting the blink startle reflex consisted of 50-ms bursts of 100-dB white noise with near instantaneous rise time, generated using the Audacity software package. To reduce predictability, startle probes were presented at varying points during video scenes. Six of the 12 scenes included 3 startle probes each, presented near the beginning (i.e., time 10 s), middle (25 s) and end (45 s) of the video. Two scenes included two startle probes each, and another two scenes included one startle probe, presented either at the beginning, middle, or end of the scene. The remaining two scenes did not include any probes. Participants heard 24 startle-probes, equally distributed across each video category.

Electromyography (EMG). EMG signals for the orbicularis oculi (ORB) muscle were sampled at 1000 Hz using two miniature Ag/AgCl electrodes filled with electrode gel and positioned over the ORB muscle under the left eye, using the guidelines of Fridlund and Cacioppo (1986). Raw EMG was rectified and integrated using a 10-ms time constant. Startle blink amplitude was scored off-line by identifying peak EMG deflections (μV) within a time window of 20-120 ms following each startle noise probe. Responses that could not be visually distinguished from baseline activity or occurred outside the post-probe 20-120 ms window were scored as missing. Mean baseline orbicularis occuli EMG activity was quantified as the mean activity across the 25ms interval preceding each startle probe and was

subtracted from the peak amplitude occurring within the 20-120 ms scoring window following noise probe onset. To establish a common metric for all participants as in prior work (e.g., Vaidyanathan, Patrick, & Bernat, 2009), raw startle magnitude values were converted to T-scores units by standardizing raw values across trials within each participant. T-scores were then averaged to represent startle magnitude values within each video category (violent and neutral).

Heart rate (HR). HR data were acquired using the electrocardiogram (ECG) module of the Biopac system. ECG was recorded using two 11-mm disposable Ag/AgCl pre-gelled electrodes placed on the right and left inner forearms of the participant. The ECG signal was amplified with a gain of 500, filtered using a Biopac ECG100C bioamplifier, and sampled online at 1000Hz, then converted offline to beats per minute values. HR was measured during a baseline period consisting of a 60-s interval preceding experiment onset (i.e., baseline HR), and during the presentation of affective and neutral video stimuli (i.e., HR activity). All scenes lasted 60 seconds each and HR was averaged for each scene and then for each affective category (negative and neutral). HR reactivity was computed by subtracting mean HR for neutral scenes from averaged HR from violent scenes

Skin conductance (SC). SC (in microSiemens, μS) was measured using two 11-mm disposable pre-gelled Ag/AgCl electrodes placed adjacently on the hypothenar eminence of the palmar surface of the non-dominant hand. The signal was amplified with a gain of 10 μS /V and sampled online at 250 Hz. SC response amplitudes were quantified as the mean conductance level during the 60-s baseline period preceding experiment onset (i.e., baseline SC), and during the presentation of affective and neutral video stimuli (i.e., SC activity). Similar to HR, SC was averaged for each scene and then was averaged for each affective category (negative and neutral). SC reactivity was computed by subtracting mean neutral SC for neutral scenes from mean SC during violent scenes.

Oxygenated Hemoglobin (HbO2). The fNIR100B is a stand-alone functional brain imaging system that includes a control device and 16-CH sensor for continuous functional Near Infrared Resonance (fNIR) spectroscopy (NIRS). Data were acquired at the standard acquisition rate of 2 Hz. The sensor consists of four LED light sources and 10 detectors, which cover the forehead using 16 voxels. The fNIR sensor detects oxygen levels in the prefrontal cortex and provides oxygenated (HbO2) and deoxygenated (HhB) hemoglobin measures, in real-time according to the modified Beer-Lambert Law. Data were collected using the COBI studio and were analyzed using the fNIRSOFT analysis software. For our study, and based on prior work (Fanti et al., 2016c), we focused solely on the oxygenated hemoglobin measure (HbO2), since HbO2 has a higher signal amplitude, is more consistent and more sensitive than HhB hemoglobin (e.g., Monden et al., 2012; Strangman, Boas, & Sutton, 2002). Coverage and data quality across the middle six sensors was good across all participants. However, due to hair obscuring full contact of the sensors at the far left and far right, data quality was not good, and thus we excluded data from these sensors from all subsequent analyses. Data from the middle six sensors (resulting in 12 voxels) comprises coverage primarily over the superior frontal and middle frontal gyrus. Upon descriptive examination of pilot data, we observed that the signal across all voxels was highly correlated with each other. Due to lack of independence between the sensors, data across sensors were pooled in second-level random-effects analyses, and from here on we will refer to collected data as data from the mPFC.

Plan of Analysis

Correlational analyses were initially used to test associations between continuous variables and physiological measures. Analysis of Variance (ANOVA) was used to test the stability of the identified groups four years later. To address our main aim, we used multivariate analyses of variance (MANOVA) to test for main effects of identified groups

with physiological measures as dependent variables and ANOVA to compare the groups on mPFC functioning. For all analysis, except for baseline measures, difference scores were computed by subtracting physiological scores during neutral scenes from negative scenes (i.e., reactivity). We opted to follow this procedure since neutral scenes were more equivalent to violent scenes, as oppose to comparing participants reactions to a baseline condition during which no stimuli were presented (Fanti et al., 2017a). Further, this was done in accord with prior studies that used an "index" (i.e., specific affect minus neutral; e.g., Miller, Patrick, & Levenston, 2002) to investigate responses to specific contents in relation to what would be considered a baseline emotion. Partial eta squares (η^2 = .01-.06 small effect size, η^2=.06-.14 medium effect size, η^2>.14 large effect size; Cohen, 1988) are reported in the text.

Study 1 Results

Correlations

Table 1 reports bivariate correlations among the main study's variables. Anxiety was correlated with CP (r =.35, p<.001), although no correlation between CU traits and anxiety was revealed. As expected, CP were significantly correlated with CU traits (r =.42, p<.001). Regarding correlations with physiological measures, findings were mostly as expected with anxiety being positively correlated with HR, SC, startle, and mpFC activity, whereas CP and CU traits were mostly negatively correlated with these measures. However, only some associations reached significance, with several correlations approaching significance. CP were negatively associated with baseline HR and startle, whereas CU traits were negatively correlated with startle.

Group comparisons

Behavioral measurements. A one-way between-groups analysis of variance was conducted to explore the continuity in levels of anxiety, CP, and CU traits among the four groups four years later, as reported in Table 2. A statistical significant difference on levels of

anxiety was identified. Post-hoc comparisons using Tukey HSD test indicated that the mean scores of Low and Primary groups were lower compared to both anxious and secondary groups. Significant differences were also identified on levels of CP. Post hoc comparisons indicated that adults in the primary and secondary groups scored higher than those in the low risk and anxious groups. Finally, individuals in both primary and secondary psychopathy groups scored higher than the low and anxious groups on CU traits.

Physiological measurements. Findings from the MANOVA comparing the identified groups on physiological measures suggested main effects for groups, Wilks' Lambda = .65, $F(15, 221.25) = 2.45$, $p < .01$, $\eta^2 = .14$. As depicted in Table 2, no group differences were identified for baseline measures of HR and SC, as well as SC reactivity. In terms of HR reactivity, findings suggested a main effect of groups, with the secondary group scoring higher than the primary and low risk groups. A main effect of groups also explained variance in startle reactivity with both the low risk and primary groups scoring lower compared to the anxious and secondary groups. The difference between the low risk and primary groups only approached significance ($p < .10$).

mPFC activity. ANOVA findings comparing identified groups on mPFC activity are also presented in Table 2. Differences in mPFC activity pointed to an unexpected finding in that the two anxious groups reacted differently to the violent scenes, with the Anxious group showing higher mPFC activity during violent scenes compared to the Secondary group. In fact, both secondary and primary groups scored similarly on mPFC activity in response to violent stimuli.

Study 2 Methods

Procedure and participant screening

Following approval of the study by the Centre of Educational Research and Assessment (CERE) of Cyprus, the Ministry of Education and Culture, and the Cyprus

Bioethics committee, 47 private and public preschools in three provinces in Cyprus (Nicosia, Larnaca and Limassol) were randomly selected for participation in Study 2. Preschools were contacted by telephone and informed about the aims of the study. School boards that were interested to participate in the study received details about the purpose and the procedure of the study via email or fax. Parents/guardians were informed of the nature of the study and 81% consented to their child's participation. Specifically, families and schools were informed that at the first stage of the study, parents will complete a battery of questionnaires and at the second phase some children will be selected to participate in an experiment involving physiological data collection.

For the purposes of study 2, questionnaire data were collected during preschool and experimental data during primary school, one year apart. At initial assessment the sample consisted of 850 (*M* age = 5.01, *SD* = 0.95) preschool children, evenly divided between boys (*n* = 435) and girls (*n* = 415). Data were collected from both mothers and fathers, and these reports were used as screening criteria. To correspond with the groups identified in study 1, we classified participants in the four groups of interest (primary, secondary, anxious and controls) using cut-off scores corresponding to 1 SD above or below the mean on CP, CU traits and Anxiety. In total 131 participants (*M*age = 5.89, *SD* = 1.43) were randomly identified to fit the groups depicted in figure 1, and families were contacted to participate in the study. From those invited, seventy two children and their families (*M* age = 5.78, *SD* = 1.33; 39 males; primary: n=18, secondary: n=18, anxious: n=18, low risk: n= 18) participated in the experiment assessing their physiological responses to affective scenes. The standard scores of the final groups (compared to the total sample) participating in the physiological part of the study are shown in Figure 2: low risk (below average on all variables), primary (high on CP and CU traits and low on anxiety), secondary (high on all three variables), and Anxious (high on anxiety). In return for their participation, families received a small

monetary reimbursement to cover their travel expenses (€15).

Questionnaires

To retain all participants, parent-reports were computed in a conservative fashion by taking the higher rating from mother and father reports, as done in prior work (Frick et al., 2003; Kyranides, Fanti, Katsimicha, & Georgiou, 2017). This method is beneficial for circumventing potential underreporting (e.g., Pardini, Lochman, & Powell, 2007) as well as handling missing data when only one informant is available. Mother and father reports of all measures assessed were moderately to highly correlated across time (r range = .51–.80). All measures were administered longitudinally across a period of one year. All self-report measures were administered in Greek.

Callous-Unemotional traits. CU traits were assessed using the 24-item preschool version of the *Inventory of Callous-Unemotional Traits* (ICU; Frick, 2004). The preschool version of the parent report aims to assess the construct of CU traits in early childhood by including items that are theoretically and empirically supported for their applicability early in development (e.g., "Does not care if he/she is in trouble. Is very expressive and emotional."). Both parents rated their children on a four point Likert scale (0 = Not at all true, 1 = Somewhat true, 2 = Very true, 3 = Definitely true) with total scores ranging from 0 to 72. Item scores of the combined variable demonstrated adequate internal consistency in the present study across time (Time 1 $\alpha = .87$; Time 2 $\alpha = .90$). Previous research has verified the validity of the ICU in samples of children in Cyprus based on parent reports (e.g., Kyranides et al., 2017; Wall, Frick, Fanti, Kimonis, & Lordos, 2016). CU traits ($r = .77$, $p < .001$) were stable across time.

Conduct Problems. The *Eyberg Child Behavior Inventory* (ECBI; Robinson, Eyberg, & Ross, 1980) is a parent scale containing 36 items, widely used to measure disruptive behaviour problems in youth between the ages of 2-16. It contains two scales, the intensity

scale (IS), which is the summed frequency of 36 symptoms, and the problem scale (PS), which reflects whether the parent perceives the specific behavior as ‘‘a problem’’. The frequency ratings range from 1 = ‘‘never’’ to 7 = ‘‘always’’ and are summed to yield an overall problem behavior IS (range 36–252). The Problem identification measure (range 0–36) is based on dichotomous ratings on each item (1 = ‘‘yes’’, it is a problem, or 0 = ‘‘no’’, it is not a problem). In the current study, only the 8 items assessing CP (Burns & Patterson, 2000) were used, and items of the combined score exhibited adequate internal consistency (Time 1 α = .77; Time 2 α = .76). This measure has already been used in a Cypriot preschool sample (Kimonis, Fanti, Anastassiou, Mertan, Goulter, & Katsimicha, 2016). CP (r = .73, p < .001) were stable across time.

Anxiety. The *Child Symptom Inventory 4* (CSI-4; Gadow & Sprafkin, 2002) is a standardized behavior rating scale designed to assess childhood disorders. Only the 8 items assessing anxiety symptoms were administered. Parents rated each CSI-4 symptom on a 4-point Likert scale ranging from 0 (never) to 3 (very often). The items were summed to create an overall Anxiety scale including items such as “Has difficulty controlling worry”, which exhibited adequate internal consistency in the current study (Time 1 α = .79; Time 2 α = .78), and high stability across time (r = .74, p < .001).This measure has been used in a Greek speaking school aged sample (Wall et al., 2016).

Experimental phase

Pilot study: To ensure that scenes elicit specific emotions an independent sample of children (N = 200; Mage = 6.13, SD = 1.25) rated an initial pool of 57 scenes from six different Disney films, on the intended content (neutral, sad, angry and fear) using emoticons. Based on these ratings a total of eight scenes, each of 1-min duration were selected and used in study 2, as the best representatives of negative valence stimuli. Scenes included video segments from the following Disney films: *Bambi (1942)* and *Aladdin (1992)*. From each

Disney film, four scenes were chosen to induce the following emotions: fear, sadness, anger and neutral. These specific affective categories were chosen based on prior work (Dadds et al., 2016) indicating different reactions of fear versus sadness in high CU children. The scenes used to elicit "fear" from Bambi show the protagonist being chased by hunters fearing for his life. In Aladdin, the protagonist is fighting the villain who has turned into a snake and is trying to kill him. The scenes that elicit "sadness" show Bambi mourning over the loss of his mother who was shot by hunters, and Jasmine crying because she thinks Aladdin has died. The scenes used to elicit "anger" show Bambi fighting with another dear and Aladdin having a heated argument with Genie. Neutral scenes which are used as comparison condition depicted scenes from the forest in Bambi and the market in Aladdin.

Experimental procedure. The participants' physiological activity in Study 2 was assessed in the same lab with the same setup used in study 1 (please refer to study 1 methods for more detailed descriptions of physiological measures and apparatus). Upon arrival at the lab, families were briefed about the procedure and provided written consent. Children were then seated in a padded reclining chair, and were fitted with the physiological sensors. The same procedures as in Study 1 were followed for assessing baseline HR and SC and HR, SC, and eye-bling activity during emotional stimuli. After the experiment, physiological sensors were removed and parents and children were debriefed.

***Experimental materials and apparatus*.** Children viewed a total of eight 60-s video scenes, which were presented in a randomized order. In addition to creating emotional categories, to follow a similar procedure as in study 1 the negative affective scenes were computed together (fear, sad, anger) and compared to neutral scenes. Similar to the selected cartoon scenes, prior work also suggested that violent scenes used among adults induce emotions of fear, sadness, and anger (Fanti et al., 2016c; Fanti et al., 2017a). All scenes were in Greek and included music and some dialog or commentary of approximately equal

duration across categories. Video soundtracks were reduced in volume such that the mean volume across each scene was 70-dB, in order to ensure that the acoustic startle probes (see below) could be easily perceived. The same apparatus used in study 1 was also used for study 2, including E-Prime 2.0 scripts for the presentation of stimuli and recording of participants' responses as well as BIOPAC MP150 for the collection of EMG, HR, and SC physiological signals. The only difference between the experiments was on the number of startle probes: Children heard a total of 12 startle probes (2 fear scenes=3 probes, 2 sadness scenes=3 probes, 2 anger scenes=3 probes and 2 neutral=3 probes), which were presented at varying points to reduce predictability, similar to study 1. Finally, the same procedures as in Study 1 were used for the assessment and computation of physiological measures.

Plan of Analysis

To be able to compare the findings across studies, the same analysis plan was followed as in Study 1 by using MANOVA to compare the identified groups on physiological activity using the combined emotional measure (i.e., combination of fear, anger, and sadness) as well as resting HR and SC. Correlational analysis also used the combined measure. Due to the use of multiple emotional stimuli, we also examined participants' responses to different types of films using repeated measures ANOVA, with the identified groups as the independent variable and physiological reactivity to different emotions as the within-subjects factor. The Greenhouse-Geisser sphericity correction was applied in evaluating repeated-measures effects. For all analysis examining individual differences, neutral films were subtracted from the corresponding affective condition. Significant interactions are depicted in figures along with standard errors. In addition to partial eta squares, standardized mean difference effect sizes (Cohen's d; d = 0.20 small effect, d = 0.50 medium effect, d = 0.80 large effect; Cohen, 1992) are reported in the text.

Study 2 Results

Correlations

Table 1 also reports bivariate correlations among the main study's variables for behavioral measures for children. Anxiety was not correlated with CP nor CU traits. CP were significantly correlated with CU traits (r =.63, p< .001). Anxiety was significantly correlated with HR and SC measures, both baseline and reactivity, during childhood, which was no identified during adulthood. CU traits were negatively correlated with startle and with SC reactivity.

Validating the Identified Groups

Behavioral measurements. A one-way between-groups analysis of variance was conducted to explore the continuity in levels of anxiety, CP, and CU traits among the four groups across a period of one year as reported in Table 3. A statistical significant difference on levels of anxiety was identified with post-hoc comparisons indicating that the mean scores of low and primary groups were lower compared to both anxious and secondary groups. Significant differences were also identified on levels of CP, with both primary and secondary groups scoring higher than the low risk group. Finally, children in both primary and secondary groups scored higher than the low and anxious groups on CU traits. Thus, the identified groups demonstrated continuity over time, providing evidence for the validity of the classification.

Physiological measures - Combined score. Findings from the MANOVA comparing the identified groups on physiological measures suggested main effects for groups, Wilks' Lambda = .54, F(15, 177.08) = 2.33, $p< .01$, $\eta^2 = .19$. As depicted in Table 3, no main effects of groups were identified for baseline SC, baseline HR, and HR reactivity. Significant findings indicated that children in the anxious group scored higher compared to everyone else on SC reactivity. As hypothesized, the primary group scored lower compared to the anxious, secondary and low risk groups on startle reactivity.

Heart rate. The findings from the repeated measures ANOVA using difference scores between emotional and neutral stimuli, showed that film-related differences in HR were significant between film types, $F(1.96, 133.22) = 10.03$, $p<.001$, $\eta^2 = .23$, $\varepsilon=.98$: Post-hoc comparisons provided evidence that sad films ($M = .07$, $SE = .31$) resulted in significantly reduced HR compared to angry ($M = 1.38$, $SE = .39$, $p<.001$) and fear films ($M = 1.19$, $SE = .36$, $p<.001$). Angry and fear films were not significantly differentiated. Beyond the basic film-related differences, we found that stratifying the sample based on the three variables under investigation revealed patterns associated with different emotion-based deficits. As Figure 3 illustrates, a significant interaction between subtypes and HR response to emotional videos was identified, $F(5.88, 133.22) = 2.96$, $p<.01$, $\eta^2 = .13$. The secondary group showed lower HR reactivity during sad compared to angry and fear stimuli ($d > 1.05$). Further, individuals in the secondary group showed similar HR activity as the primary group during sad stimuli, and lower than the anxious and low risk groups with medium effect sizes ($d = .41$-$.47$). In contrast, for angry and fear stimuli, individuals in the secondary group scored similarly as the anxious group and higher than the primary and low risk groups ($d = .68$-$.85$), pointing to distinct associations with different emotional stimuli.

Skin Conductance. For SC, the repeated measures ANOVA only suggested a significant interaction between subtypes and SC response to emotional videos, $F(5.71, 129.50) = 2.77$, $p<.05$, $\eta^2 = .12$. Similar to HR, the secondary and primary groups showed lower SC reactivity during sad stimuli compared to the Anxious group ($d > 1.10$). In contrast, both the anxious and secondary groups showed high SC reactivity to fear-related stimuli compared to the primary ($d = .72$-$.86$) and low risk ($d = .43$-$.57$) groups. The secondary group also showed higher SC reactivity to angry stimuli compared to the primary group ($d = .42$).

Startle. The repeated measures ANOVA with startle reactivity as the dependent variable only pointed to a between group effect, $F(3, 68) = 8.76$, $p<.001$, $\eta^2 = .29$, and no

interaction with specific emotions, replicating the findings reported in Table 3.

Discussion

The current study compares individuals high on anxiety with primary and secondary psychopathy groups on various neurophysiological measures across development. By integrating multiple physiological measures and taking heterogeneity into account, findings were expected to disentangle conflicting evidence from prior work. The present study contributes five key findings: First, no differences were identified in baseline SC and HR between the four groups under investigation, indicating that it is important to investigate individual differences in relation to responsiveness to emotional stimuli. Second, HR reactivity to violent, angry, and fearful stimuli differentiated primary from secondary psychopathy groups across development. Third, Anxious children showed higher SC and HR reactivity to sad stimuli compared to children in both primary and secondary groups. Forth, startle potentiation can be used as a measure to differentiate anxious individuals, irrespective of CU traits, from those in the primary psychopathy group. Finally, mPFC activity differentiated adults in the anxious group from those in the secondary group, with the anxious group showing higher mPFC activation when presented with violent stimuli and the secondary group showing low activation of the mPFC in response to these stimuli. Thus, the study's findings point to distinct associations between neuro-physiological markers with heterogeneous groups. In the majority of prior neuro-physiological studies, individuals with high levels of CP and CU traits are clustered into one group, without taking into consideration the presence of anxiety. This is unfortunate, since increasing evidence shows that primary and secondary psychopathy constitute two distinct groups (e.g., Fanti et al., 2013; Hicks et al., 2004) differentiated on multiple physiological measures.

Physiological arousal

Findings did not replicate prior evidence differentiating primary from secondary

psychopathy groups on baseline measures of arousal (e.g., Fanti & Kimonis, 2017). Regarding HR and SC reactivity to emotional stimuli, previous studies contradict one another, either proposing lower physiological reactions or no significant differences among antisocial subtypes (Fanti, 2016 for a review). These findings can be informed from current results. Our hypothesis that individuals in the primary psychopathy group would show lower physiological arousal compared to the secondary group was supported based on their HR, but not SC, reactivity in response to violent stimuli during adulthood. These findings agree with adolescent and adult conceptualizations of primary psychopathy as an emotionally unresponsive group of individuals (Hicks & Patrick, 2006; Kimonis et al., 2016)

During childhood, findings indicated that high SC reactivity differentiated Anxious individuals from all other groups on a measure combining various negative emotions. Additional analysis pointed to interactions between emotions and groups predicting both HR and SC reactivity among children. Specifically it was found that children in both primary and secondary psychopathy groups showed similar HR and SC reactivity to sad emotional stimuli, although the anxious group reacted with higher physiological arousal to this stimuli. An explanation can be offered based on the empathy model of psychopathy (Frick et al., 2014), which proposes that individuals scoring high on CU traits, regardless of anxiety, exhibit deficits in affective sharing and resonating with others' feelings. Thus, measures of physiological arousal in response to sad stimuli, which possibly relate to affective empathy (Blair, 2013), might act as biomarkers differentiating predominately anxious individuals from those high on CU traits. Moreover, an important developmental difference is that SC deficits were only identified among children, which might be due to self-regulation deficits seen more commonly in youth compared to adults (e.g., Duell et al., 2016; Wall et al., 2016). These findings suggest that physiological mechanisms might function differently across age, indicating that it is important to consider developmental differences in future studies.

Regarding anger and fear stimuli, findings indicated that the secondary group resembled the anxious group by showing high SC and HR reactivity to this stimuli. Further, the fact that both groups showed increased HR and SC in response to angry and fearful emotions is in line with prior studies which underlined that anxious individuals show higher responsiveness to threatening cues (e.g., anger or fear) (Fanti, 2016; Masten et al., 2008; Schoorl et al., 2016). Thus, high reactivity to threatening stimuli might be a potential physiological marker associated with both anxious and secondary groups. These differences in emotional processing may provide an additional method through which secondary psychopathy may be distinguished from primary psychopathy. Additionally, these findings can inform prior work and theoretical accounts proposing that the co-occurrence between CP with CU traits is related with under-arousal (e.g., Anastassiou-Hadjicharalambous & Warden, 2008; Fanti et al., 2016a; Frick et al., 2014), by suggesting that this finding might be specific to individuals in the primary psychopathy group who show low stress reactivity. Findings not taking this heterogeneity into account might have resulted in contradicting or non-significant findings.

Startle reactivity

Limited studies have also shown that individuals high on CP and CU traits are characterized with fearlessness and startle attenuation to negative stimuli (Fanti et al., 2016b; 2016c; 2017a, Kyranides et al., 2016). In line with these studies, current findings suggest that individuals in the primary psychopathy group, regardless of age and type of emotional stimuli, show lower startle reactivity compared to individuals high on anxiety with or without CU traits. Importantly, the results of the current study replicate prior work assessing startle reactivity among juvenile offenders differentiated into primary and secondary psychopathy groups based on their history of abuse (Kimonis et al., 2016). These findings lead to three interesting suggestions: First, startle modulation was the only measure that consistently

differentiated all three high-risk groups across development, pointing to a continuity of low to high startle reactivity differentiating the three groups under investigation. Second, findings suggest that anxiety, but not CU traits, might drive the identified differences, which contradicts the negative association with the continuous measure of CU traits found in current and prior work (Fanti et al., 2017a). Thus, startle reactivity can function as a physiological marker for distinguishing anxious from non-anxious antisocial individuals. Third, our findings have implications for the fearlessness theory (Raine, 1993) by providing evidence for a fear–fearless continuum, with the primary psychopathy group being on the one extreme and the secondary psychopathy and anxious groups at the other extreme. The low risk group scored in the middle of this continuum, although differences with the primary and secondary psychopathy groups did not always achieve significance. Notably, both children and adults in the secondary and anxious groups showed physiological responses associated with high levels of fearfulness and negative affectivity.

mPFC activity

Our hypothesis for increased mPFC activation in both anxious and secondary psychopathy groups was partially supported. In accordance with prior work demonstrating that anxiety is associated with over-activity in mPFC during threatening or empathy related conditions (Etkin et al., 2011; Fanti, 2016; Posner et al., 2009), the anxious group showed high mPFC activity in response to violent stimuli. However, this was not true for all individuals scoring high on anxiety, with the secondary group showing low mPFC activation in response to violent stimuli or seeing others (i.e., victims of violence) in distress, which was similar to the primary group. Current results might be explained by findings from a meta-analysis suggesting that activity in the mPFC mediates human empathy (Seitz, Nickel, & Azari, 2006). Based on the study's findings, we can conclude that individuals with CU traits show low mPFC activity in response to stimuli depicting violent interactions due to their low

empathy and guilt (Fanti et al., 2016a). However, anxious individuals with no deficits in empathy might indeed be over-reacting to this emotional information. Additionally, the reduced mPFC activation in both primary and secondary psychopathy groups is in line with existing literature which portrays a negative association between CU traits with activity of brain areas involved in emotional processing in response to negative affective stimuli (Anderson & Stanford, 2012; Blair, 2013; Fanti et al., 2016a). As a result its seems that mPFC activation differences poses a risk contributing to these traits. Overall, our results indicate that CU traits and CP can be perceived as risk factors for the under-functioning of the mPFC during processing of emotional information, whereas anxiety in the absence of CU traits might be associated with increased mPFC activation.

Clinical Implications

The neuro-physiological measurements used in the current study might be important in identifying the underlying mechanisms associated with impairments in emotional processing, among adults and children with different levels of CU traits, CP and anxiety (Fanti, 2016). Such findings could improve the effectiveness of interventions as they can shed light to the question as to why some individuals are less responsive to treatment. For instance, anxious individuals or secondary psychopathy groups might respond better to traditional interventions focusing on anxiety and distress reduction or emotional regulation. On the contrary, reward-based interventions might be more successful for individuals in the primary psychopathy group that tend to be under-aroused and in need of stimulation (Dadds, Cauchi, Wimalaweera, Hawes, & Brennan, 2012; Fanti, 2016; Fanti et al., 2013; Kimonis & Armstrong, 2012).

Findings related to mPFC activity and physiological responses to sad stimuli indicate that interventions focusing on enhancing empathy should target both primary and secondary psychopathy groups. Although, it is possible that early interventions administered during

childhood might result in better outcomes (Kyranides et al., 2017), our findings suggest that both adults and children showing the same phenotypic profile might be benefitted from similar interventions. Additionally, by appreciating the role of anxiety in influencing emotional processing, it is likely to enhance the field's understanding of the development, maintenance and treatment of CU traits, which might be relevant for discontinuing the developmental cycle leading to severe antisocial behavior during the lifespan (Fanti, 2016; Fanti & Kimonis, 2017; Frick et al., 2014).

Strengths, Limitations and Future Directions

Current findings should be interpreted within the context of some limitations. Firstly, the study's phenotypic indicators were assessed by self- and parent-reports. Future studies should incorporate clinical interviews as an additional assessment for identifying each profile. Secondly, we examined differences in emotional processing using physiological measurements in community samples of adults and children. Future work investigating these research questions in clinical samples should be conducted. Thirdly, it will be useful to examine the contribution of history of trauma and victimization to the development of antisocial behavior, especially in the secondary group, which might inform their affective and physiological responses. Therefore, the above suggestions may shed more light on distinguishing further the similarities and differences between primary and secondary psychopathy groups. Fourth, mPFC assessment was only available for adults, and it will be interesting to investigate whether the same differences can be identified among children. Fifth the videos presented to adults were in English while the videos presented to children were in Greek. This change in audio language was done for children as not all children spoke fluent English.

The study has several strengths, including a multi-method physiological assessment, measuring arousal (HR, SC), valence (startle reflex), and mPFC activity, to identify

differences in the physiological reactivity of anxious, primary, and secondary groups. Moreover, large community samples were used for the identification of individuals in each profile under investigation. An additional strength is the examination of developmental continuity, since a prospective longitudinal design was used to follow preschoolers into childhood and adolescents into adulthood. The study's design also enabled the comparison of children with adults and testing the continuity of physiological reactivity in response to affective stimuli. Lastly, we used age appropriate emotional scenes which are considered more realistic, empowering the generalization of emotion processing deficits in CU traits, CP and anxiety in both adult and children populations.

In conclusion, our findings highlight the importance of different cortical and physiological markers in understanding the emotional processing of individuals distinguished on levels of CU traits, CP, and anxiety. Finding differences on biological and physiological vulnerability can inform efforts toward identifying research domain criteria of psychological disorders (Insel et al. , 2010) and can help shape etiological hypotheses to address in future research (Fanti, 2016). Models explaining antisocial behavior in relation to anxiety and CU traits need to consider heterogeneity at the neuro-physiological level, especially in relation to different reactions to distinct emotional stimuli (i.e., sad versus fear). Although our results propose that developmental differences might be important for understanding some of these associations, findings also point to developmental continuity in physiological reactions from childhood to adulthood. Taking into consideration differences in emotional reactivity can contribute to interventions designed specifically for anxious individuals, primary or secondary psychopathy groups, leading to higher treatment efficacy.

References

Anastassiou-Hadjicharalambous, X., & Warden, D. (2008). Physiologically-indexed and self-perceived affective empathy in conduct-disordered children high and low on callous-unemotional traits. *Child Psychiatry and Human Development, 39*(4), 503-517.

Anderson, N. E., & Stanford, M. S. (2012). Demonstrating emotional processing differences in psychopathy using affective ERP modulation. *Psychophysiology*, *49*(6), 792-806.

Bagley, A. D., Abramowitz, C. S., & Kosson, D. S. (2009). Vocal affect recognition and psychopathy: Converging findings across traditional and cluster analytic approaches to assessing the construct. *Journal of Abnormal Psychology*, *118*(2), 388-398.

Balconi, M., Bortolotti, A., & Gonzaga, L. (2011). Emotional face recognition, EMG response, and medial prefrontal activity in empathic behaviour. *Neuroscience research*, *71*(3), 251-259.

Beauchaine, T. P. (2012). Physiological markers of emotion and behavior dysregulation in externalizing psychopathology. *Monographs of the Society for Research in Child Development, 77*(2), 79-86.

Blair, R. J. R. (1995). A cognitive developmental approach to morality: Investigating the psychopath. *Cognition, 57*, 1–29.

Blair, R. J. (2013). The neurobiology of psychopathic traits in youths. *Nature Reviews Neuroscience*, *14*(11), 786-799.

Blair, R. J. R., Jones, L., Clark, F., & Smith, M. (1997). The psychopathic individual: A lack of responsiveness to distress cues? *Psychophysiology, 34,* 192–198.

Blair, R. J. R., Mitchell, D. G. V., Peschardt, K. S., Colledge, E., Leonard, R. A., Shine, J. H., Murray, L. K., & Perrett, D. I. (2004). Reduced sensitivity to others' fearful expressions in psychopathic individuals. *Personality and Individual Differences*, *37*(6), 1111-1122.

Burns, G. L., & Patterson, D. R. (2000). Factor structure of the Eyberg Child Behavior Inventory: A parent rating scale of oppositional defiant behavior toward adults, inattentive behavior, and conduct problem behavior. *Journal of Clinical Child Psychology*, *29*, 569-577.

Cleckley, H. M. (1941). *The mask of sanity: An attempt to reinterpret the so-called psychopathic personality*. St. Louis: The C.V. Mosby Company.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Cohen, J. (1992). A power primer. Psychological Bulletin, 112, 155–159.

Dadds, M. R., Cauchi, A. J., Wimalaweera, S., Hawes, D. J., & Brennan, J. (2012). Outcomes, moderators, and mediators of empathic-emotion recognition training for complex conduct problems in childhood. *Psychiatry research*, *199*(3), 201-207.

Dadds, M. R., Kimonis, E. R., Schollar-Root, O., Moul, C., & Hawes, D. (2017). Are impairements in emotion recognition a core feature of callous-unemotional traits? Testing the primary versus secondary variants model in children. *Development and Psychopathology,* 1-11.

Dackis, M. N., Rogosch, F. A., & Cicchetti, D. (2015). Child maltreatment, callous–unemotional traits, and defensive responding in high-risk children: An investigation of emotion-modulated startle response. *Development and Psychopathology*, 27, 1527-1545.

de Wied, M., van Boxtel, A., Matthys, W., & Meeus, W. (2012). Verbal, facial and autonomic responses to empathy-eliciting film clips by disruptive male adolescents with high versus low callous-unemotional traits. *Journal of Abnormal Child Psychology*, *40*(2), 211-223.

Drislane, L. E., Patrick, C. J., Sourander, A., Sillanmäki, L., Aggen, S. H., Elonheimo, H.,

Parkkola, K., Multimäki, P., & Kendler, K. S. (2014). Distinct variants of extreme psychopathic individuals in society at large: Evidence from a population-based sample. *Personality Disorders: Theory, Research, and Treatment, 3*(2), 154-163.

Duell, N., Steinberg, L., Chein, J., Al-Hassan, S.M., Takash, H., Bacchini, D., et al. (2016). Interaction of reward seeking and self-regulation in the prediction of risk taking: A Cross-National Test of the Dual Systems Model. *Developmental Psychology*, *52*(10), 1593-1605.

Etkin, A., Egner, T., & Kalisch, R. (2011). Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends in cognitive sciences*, *15*(2), 85-93.

Fanti, K. A. (2016). Understanding heterogeneity in conduct disorder: A review of psychophysiological studies. *Neuroscience & Biobehavioral Reviews.* doi: 10.1016/j.neubiorev.2016.09.022

Fanti, K. A., Demetriou, C. A., & Kimonis, E. R. (2013). Variants of callous-unemotional conduct problems in a community sample of adolescents. *Journal of Youth and Adolescence, 42*(7), 964-979.

Fanti, K. A., Frick, P. J., & Georgiou, S. N. (2008). Linking callous-unemotional traits to instrumental and non-instrumental forms of aggression. *Journal of Psychopathology and Behavioral Assessment, 31*(4), 285-298.

Fanti, K. A., & Henrich, C. C. (2010). Trajectories of pure and co- occurring internalizing and externalizing problems from age 2 to age 12: Findings from the National Institute of Child Health and Human Development Study of Early Child Care. *Developmental Psychology, 46*(5), 1159–1175.

Fanti, K. A. & Kimonis, E. (2017). Primary and secondary variants at age 3: Associations with age 15 biological and environmental variables. *Developmental Psychology, 53*(7), 1230-1241. doi: 10.1037/dev0000317

Fanti, K. A., Kyranides, M. N., Georgiou, G., Petridou, M., Colins, O. F., Tuvblad, C., & Andershed, H. (2017a). Callous-unemotional, impulsive-irresponsible, and grandiose-manipulative traits: Distinct associations with heart rate, skin conductance, and startle responses to violent and erotic scenes. *Psychophysiology*, 54(5), 663-672.

Fanti, K. A., Kyranides, M. N., & Panayiotou, G. (2017b). Facial reactions to violent and comedy films: Association with callous–unemotional traits and impulsive aggression. *Cognition and Emotion*, *31*, 209-224.

Fanti, K. A., Panayiotou, G., Kyranides, M. N., & Avraamides, M. N. (2016a). Startle modulation during violent films: Association with callous–unemotional traits and aggressive behavior. *Motivation and Emotion*, *40*(2), 321-333.

Fanti, K.A., Panayiotou, G., Lazarou, C., Michael, R., & Georgiou, G. (2016b). The better of two evils? Evidence that children exhibiting continuous conduct problems high or low on callous–unemotional traits score on opposite directions on physiological and behavioral measures of fear. *Development and Psychopathology, 28*(1), 185-198.

Fanti, K.A., Panayiotou, G., Lombardo, M., & Kyranides, M.N. (2016c). Unemotional on all counts: Evidence of reduced affective responses in individuals with high callous-unemotional across emotion systems and valences. *Social Neuroscience, 11*, 72-87.

Frick, P.J. (2004). *Inventory of callous–unemotional traits*. Unpublished rating scale, University of New Orleans, New Orleans, LA.

Frick, P. J., Cornell, A. H., Bodin, S. D., Dane, H. E., Barry, C. T., & Loney, B. R. (2003). Callous unemotional traits and develop- mental pathways to severe conduct problems. *Developmental Psychology, 39*(2), 246–260.

Frick, P. J., & Morris, A. S. (2004). Temperament and developmental pathways to conduct problems. *Journal of Clinical Child and Adolescent Psychology, 33*, 54-68.

Frick, P. J., Ray, J. V., Thornton, L. C., & Kahn, R. E. (2014). Annual research review: a developmental psychopathology approach to understanding callous-unemotional traits in children and adolescents with serious conduct problems. *Journal of Child Psychology and Psychiatry, 55*, 532–548.

Frick, P. J., & White, S. F. (2008). Research review: The importance of callous- unemotional traits for developmental models of aggressive and antisocial behavior. *Journal of Child Psychology and Psychiatry*, *49*(4), 359-375.

Gadow, K. D., & Sprafkin, J. N. (1999). *Youth's Inventory 4 manual.* Stony Brook, NY: Checkmate Plus.

Gadow, K. D., & Sprafkin, J. N. (2002). *Child symptom inventory 4: Screening and norms manual*. Stony Brook, NY: Checkmate Plus.

Grillon, C., & Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology*, *114*(9), 1557-1579.

Hare, R. D. (2003). *Hare Psychopathy Checklist-Revised (PCL-R): 2nd edition, technical manual.* Toronto: Multi-Health Systems Inc.

Hicks, B. M., Markon, K. E., Patrick, C. J., Krueger, R. F., & Newman, J. P. (2004). Identifying psychopathy subtypes on the basis of personality structure. *Psychological Assessment, 16,* 276–288.

Hicks, B. M., & Patrick, C. J. (2006). Psychopathy and negative emotionality: Analyses of suppressor effects reveal distinct relation with emotional distress, fearfulness, and anger-hostility. *Journal of Abnormal Psychology, 115*, 276–287

Hoehn-Saric, R., McLeod, D. R., Funderburk, F., & Kowalski, P. (2004). Somatic symptoms and physiologic responses in generalized anxiety disorder and panic disorder: An ambulatory monitor study. *Archives of General Psychiatry*, *61*(9), 913-921.

Huesmann, L. R., Dubow, E., & Boxer, P. (2009). Continuity of aggression from childhood

to early adulthood as a predictor of life outcomes: Implications for the adolescent limited and life course persistent models. *Aggressive Behavior*, *35*, 136-149.

Insel, T. R., Cuthbert, B. N., Garvey, M. A., Heinssen, R. K., Pine, D. S., Quinn, K. J., Sanislow, C., & Wang, P. (2010). Research domain criteria (RDoC): Toward anew classification framework for research on mental disorders. *American Journal of Psychiatry, 167,* 748–751.

Kahn, R. E., Frick, P. J., Youngstrom, E. A., Kogos Youngstrom, J., Feeny, N. C., & Findling, R. L. (2013). Distinguishing primary and secondary variants of callous-unemotional traits among adolescents in a clinic-referred sample. *Psychological Assessment, 25,* 966–978.

Karpman, B. (1941). On the need of separating psychopathy into two distinct clinical types: The symptomatic and the idiopathic. *Journal of Criminology and Psychopathology, 3,* 112–137.

Karpman, B. (1948a). Conscience in the psychopath: Another version. *American Journal of Orthopsychiatry, 18*(3), 455–491.

Karpman, B. (1948b). The myth of the psychopathic personality. *American Journal of Psychiatry, 104,* 523–534.

Kaviani, H., Gray, J. A., Checkley, S. A., Raven, P. W., Wilson, G. D., & Kumari, V. (2004). Affective modulation of the startle response in depression: influence of the severity of depression, anhedonia, and anxiety. *Journal of affective disorders*, *83*(1), 21-31.

Kimonis, E. R., & Armstrong, K. (2012). Adapting parent–child interaction therapy to treat severe conduct problems with callous-unemotional traits: A case study. *Clinical Case Studies*, *11*(3), 234-252.

Kimonis, E. R., Fanti, K. A., Anastassiou-Hadjicharalambous, X., Mertan, B., Goulter, N., & Katsimicha, E. (2016). Can callous-unemotional traits be reliably measured in

preschoolers? *Journal of abnormal child psychology*, *44*, 625-638.

Kimonis, E. R., Fanti, K. A., Goulter, N., & Hall, J. (2016). Affective startle potentiation differentiates primary and secondary variants of juvenile psychopathy. *Development & Psychopathology, 29,* 1-12.

Kimonis, E. R., Frick, P. J., Cauffman, E., Goldweber, A., & Skeem, J. (2012). Primary and secondary variants of juvenile psychopathy differ in emotional processing. *Development and Psychopathology*, *24*(3), 1091-1103.

Kimonis, E. R., Frick, P. J., Fazekas, H., & Loney, B. R. (2006). Psychopathy, aggression, and the processing of emotional stimuli in non-referred girls and boys. *Behavioral Sciences & The law*, *24*(1), 21-37

Kimonis, E. R., Frick, P. J., Muñoz, L. C., & Aucoin, K. J. (2008). Callous–unemotional traits and the emotional processing of distress cues in detained boys: Testing the moderating role of aggression, exposure to com- munity violence, and histories of abuse. *Development and Psychopathology, 20,* 569–589.

Kimonis, E. R., Skeem, J. L., Cauffman, E., & Dmitrieva, J. (2011). Are secondary variants of juvenile psychopathy more reactively violent and less psychosocially mature than primary variants? *Law and Human Behavior, 35,* 381–391.

Kosson, D. S., Suchy, Y., Mayer, A. R., & Libby, J. (2002). Facial affect recognition in criminal psychopaths. *Emotion*, *2*(4), 398-411.

Kramer, M. D., Patrick, C. J., Krueger, R. F., & Gasperi, M. (2012). Delineating physiologic defensive reactivity in the domain of self-report: phenotypic and etiologic structure of dispositional fear. *Psychological medicine*, *42*(06), 1305-1320.

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: the impact of age and

gender. *International journal of behavioral medicine*, *11*(2), 116-121.

Kyranides, M. N., Fanti, K. A., Katsimicha, E., & Georgiou, G. (2017). Preventing conduct disorder and callous-unemotional traits: Preliminary results of a school based pilot training program. *Journal of Abnormal Child Psychology*. DOI: 10.1007/s10802-017-0273-x

Kyranides, M. N., Fanti, K. A., & Panayiotou, G. (2015). The Disruptive Adolescent as a Grown-Up: Predicting Adult Startle Responses to Violent and Erotic Films from Adolescent Conduct Problems and Callous-Unemotional Traits. *Journal of Psychopathology and Behavioral Assessment*, *38*(2), 183-194.

Lang, P., Greenwald, M., Bradley, M., & Hamm, A. (1993). Looking at pictures: Affective, facial, visceral, and behavioral reactions. *Psychophysiology*, *30*, 261–273.

Leist, T., & Dadds, M. R. (2009). Adolescents' ability to read different emotional faces relates to their history of maltreatment and type of psychopathology. *Clinical Child Psychology and Psychiatry, 14*(2), 237-250.

Lorber, M. F. (2004). Psychophysiology of aggression, psychopathy, and conduct problems: a meta-analysis. *Psychological Bulletin, 130*(4), 531-552.

Lykken, D. T. (1995). *The antisocial personalities*. Hillsdale, NJ: Lawrence Erlbaum.

Masten, C. L., Guyer, A. E., Hodgdon, H. B., McClure, E. B., Charney, D. S., Ernst, M., Kaufman, J., Daniel, S., P., & Monk, C. S. (2008). Recognition of facial emotions among maltreated children with high rates of post-traumatic stress disorder. *Child abuse & Neglect, 32*(1), 139-153

Mealey, L. (1995). The sociobiology of sociopathy: An integrated evolutionary model. *Behavioral and Brain sciences*, *18*(3), 523-541.

Mezzacappa, E., Tremblay, R. E., Kindlon, D., Saul, J. P., Arseneault, L., Seguin, J., Robert, O., & Earls, F. (1997). Anxiety, antisocial behavior, and heart rate regulation in

adolescent males. *Journal of Child Psychology and Psychiatry*, 38(4), 457-469.

Miller, M. W., & Patrick, C. J. (2000). Trait differences in affective and attentional responding to threat revealed by emotional Stroop interference and startle reflex modulation. *Behavior Therapy*, *31*(4), 757-776.

Miller, M. W., Patrick, C. J., & Levenston, G. K. (2002). Affective imagery and the startle response: Probing mechanisms of modulation during pleasant scenes, personal experiences, and discrete negative emotions. *Psychophysiology, 39*(4), 519–529.

Moffitt, T. E. (1993). Adolescence-limited and life-course persistent antisocial behavior: A developmental taxonomy. *Psychological Review, 100,* 674–701.

Monden, Y., Dan, H., Nagashima, M., Dan, I., Tsuzuki, D., Kyutoku, Y., Gunji, Y., Yamagata, T., Watanabe, E., & Momoi, M. Y. (2012). Right prefrontal activation as a neuro-functional biomarker for monitoring acute effects of methyl-phenidate in ADHD children: An fNIRS study. *NeuroImage: Clinical, 1,* 131–140.

National Collaborating Centre for Mental Health (2013). *Antisocial behaviour and conduct disorders in children and young people: recognition, intervention and management* (Vol. 158). Royal College of Psychiatrist: London.

Northover, C., Thapar, A., Langley, K., & van Goozen, S. H. (2015). Pain Sensitivity in Adolescent Males with Attention-Deficit/Hyperactivity Disorder: Testing for Associations with Conduct Disorder and Callous and Unemotional Traits. *PloS one*, *10*(7).

Pardini, D. A., Lochman, J. E., & Powell, N. (2007). The development of callous-unemotional traits and antisocial behavior in children: Are there shared and/or unique predictors? *Journal of Clinical Child and Adolescent Psychology, 36*(3), 319-333.

Patrick, C. J. (1994). Emotion and psychopathy: Startling new insights. *Psychophysiology*, *31*(4), 319-330.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*, *16*(2), 331-348.

Porter ME. 1996. What is strategy? *Harvard Business Review 74*(6): 61–78.

Posner, J., Russell, J. A., Gerber, A., Gorman, D., Colibazzi, T., Yu, S., Wang, Z., Kangarlu, A., Zhu, H., & Peterson, B. S. (2009). The neurophysiological bases of emotion: An fMRI study of the affective circumplex using emotion- denoting words. *Human brain mapping*, *30*(3), 883-895.

Posner, J., Russell, J. A., & Peterson, B. S. (2005). The circumplex model of affect: An integrative approach to affective neuroscience, cognitive development, and psychopathology. *Development and Psychopathology*, *17*(3), 715-734.

Porter, S. (1996). Without conscience or without active conscience? The etiology of psychopathy revisited. *Aggression and Violent Behavior, 1*, 179-189.

Raine, A. (1993). The psychopathology of crime: Criminal behavior as a clinical disorder. *San Diego, CA: Academic Press.*

Richell, R. A., Michell, D. G. V., Peschardt, K. S., Winston, J. S., Leonard, A., Dolan, R. J., Blair, R. J. R. (2005). Trust and distrust: The perception of trustworthiness of faces in psychopathic and non- psychopathic offenders. *Personality and Individual Differences, 38(8),* 1735–1744.

Robinson, E. A., Eyberg, S. M., & Ross, A. W. (1980). The standardization of an inventory of child conduct problem behaviors. *Journal of Clinical Child & Adolescent Psychology*, *9*(1), 22-28.

Rogeness, G. A., Cepeda, C., Macedo, C. A., Fisher, C., & Harris, W. R. (1990). Differences in heart rate and blood pressure in children with conduct disorder, major depression, and separation anxiety. *Psychiatry Research,* 33(2), 199-206.

Rosebrock, L. E., Hoxha, D., Norris, C., Cacioppo, J. T., & Gollan, J. K. (2016). Skin Conductance and Subjective Arousal in Anxiety, Depression, and Comorbidity. *Journal of Psychophysiology,* DOI: 10.1027/0269-8803/a000176.

Schneider, W., Eschmann, A., & Zuccolotto, A. (2002). *E-Prime reference guide*. Pittsburgh, PA: Psychology Software Tools.

Schoorl, J., Van Rijn, S., De Wied, M., Van Goozen, S. H., & Swaab, H. (2016). Variability in emotional/behavioral problems in boys with oppositional defiant disorder or conduct disorder: the role of arousal. *European Child & Adolescent Psychiatry, 25*(8), 821-830.

Scott, S., Knapp, M., Henderson, J., & Maughan, B. (2001). Financial cost of social exclusion: follow up study of antisocial children into adulthood. *Bmj*, *323*(7306), 191.

Seitz, R. J., Nickel, J., & Azari, N. P. (2006). Functional Modularity of the Medial Prefrontal Cortex. *Neuropsychology*, *20*(6), 743-751.

Strangman, G., Boas, D. A., & Sutton, J. P. (2002). Non-invasive neuroimaging using near-infrared light. *Biological Psychiatry, 52*(7), 679-693.

Vaidyanathan, U., Patrick, C. J., & Bernat, E. M. (2009). Startle reflex potentiation during aversive picture viewing as an indicator of trait fear. *Psychophysiology*, *46*(1), 75-85.

Vaidyanathan, U., Patrick, C. J., & Cuthbert, B. N. (2009). Linking dimensional models of internalizing psychopathy to neurobiological systems: affect-modulated startle as an indicator of fear and distress disorders and affiliated traits. *Psychological Bulletin, 135*(6), 909-942.

Verona, E., Patrick, C. J., & Joiner, T. E. (2001). Psychopathy, antisocial personality, and suicide risk. *Journal of Abnormal Psychology, 110,* 462–470.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: a new measure of emotion? *Journal of abnormal psychology*, *97*(4), 487-491.

Wager, T. D., van Ast, V. A., Hughes, B. L., Davidson, M. L., Lindquist, M. A., & Ochsner, K. N. (2009). Brain mediators of cardiovascular responses to social threat, part II: Prefrontal-subcortical pathways and relationship with anxiety. *Neuroimage*, *47*(3), 836-851.

Wall, T. D., Frick, P. J., Fanti, K. A., Kimonis, E. R., & Lordos, A. (2016). Factors differentiating callous- unemotional children with and without conduct problems. *Journal of Child Psychology and Psychiatry*, *57*, 976-983.

Williamson, S., Harpur, T. J., & Hare, R. D. (1991). Abnormal processing of affective words by psychopaths. *Psychophysiology, 28*(3), 260–273.

Woodworth, M., & Waschbusch, D. (2008). Emotional processing in children with conduct problems and callous/unemotional traits. *Child: Care, Health and Development, 34*, 234-244.

Table1*: Correlations among the main study variables assessed during the experimental parts of studies 1 and 2.*

	Anxiety	Conduct problems	CU traits
Anxiety	_____	.06[b]	.04[b]
Conduct problems	**.35[a]**	_____	**.63[b]**
CU traits	.07[a]	**.42[a]**	_____
Study 1- Adults			
HR (baseline)	.16	**-.28**	-.03
SC (baseline)	.17	-.14	-.05
HR reactivity	.16	-.13	-.06
SC reactivity	.11	-.04	-.15
Startle reflex	.10	-.20*	**-.27**
mPFC	.12	-.14	-.13
Study 2 - Children			
HR (baseline)	.24*	-.17	-.08
SC (baseline)	.28*	.06	-.12
HR reactivity	.29*	-.17	-.10
SC reactivity	**.37**	-.12	-.20*
Startle reflex	.09	.05	-.19*

Note: Two-tailed significance: * entries are significant at $p<.05$ and bold font entries are significant at the $p<.01$ level. Different subscripts ([a,b]) denote different age groups: ([a]) adults and ([b]) children.

Table 2. *Comparisons between the Identified Groups among adults (Study 1)*

	Low risk (n = 40)	Anxious (n = 38)	Primary (n = 41)	Secondary (n = 41)	F-value	df	η^2
Continuity							
Anxiety	4.59(.72)[a]	9.06(.72)[b]	4.85(.79)[a]	8.99(.68)[b]	11.63**	3	.36
Conduct problems	2.64(1.65)[a]	2.82(1.65)[a]	9.37(1.56)[b]	10.36(1.82)[b]	6.02**	3	.22
CU traits	15.91(1.97)[a]	19.83(2.23)[a]	28.01(2.02)[b]	29.09(2.02)[b]	10.05**	3	.27
Baseline physiology							
HR	81.10(1.91)	80.32(2.16)	79.90(1.99)	76.65(1.96)	.98	3	.04
SC	8.28(1.87)	7.18 (2.11)	8.14(1.96)	8.12(1.87)	.06	3	.00
Emotional-Physiology							
(violent vs neutral)							
HR reactivity	-.61(.66)[a]	-.38(.75)[ab]	-.89(.69)[a]	1.59(.68)[b]	2.70*	3	.09
SC reactivity	.41(.11)	.11(.13)	.01(.12)	.08(.12)	2.24	3	.08
Startle reactivity	3.03(2.62)[a]	11.38(2.91)[b]	-3.04(2.69)[a]	12.03(2.62)[b]	7.06**	3	.21
mPFC	.13(.23)[ab]	.60(.25)[b]	-.20(.23)[ab]	-.50(.24)[a]	3.83*	3	.13

Note: Estimated marginal means (SE)*;* Difference scores denote scores minus neutral. Different subscripts ([a,b,c]) denote significant differences between groups in post hoc pairwise comparisons. Values from the experimental phase are reported in the Table.

Table 3. *Comparisons between the Identified Groups among children (Study 2)*

	Low risk (n = 40)	Anxious (n = 38)	Primary (n = 41)	Secondary (n = 41)	F-value	df	η^2
Children							
Anxiety	.83(.83)[a]	2.61(.98)[b]	1.21(.92)[a]	2.36(1.50)[b]	8.83**	3	.29
Conduct problems	40.01(2.65)[a]	41.55(3.54)[ab]	48.74(4.06)[b]	47.31(5.67)[b]	3.49*	3	.24
CU traits	13.87(1.68)[a]	17.71(2.21)[a]	25.05(1.90)[b]	24.47(2.13)[b]	8.53**	3	.28
Baseline physiology							
Child HR	89.29(3.11)	90.04(2.72)	88.21(2.25)	90.54(2.45)	.18	3	.01
Child SC	13.05(5.13)	12.15 (4.72)	13.69(3.91)	22.01(4.26)	1.04	3	.05
Emotional-Physiology (negative vs neutral)							
Child HR reactivity	.77(.77)	1.10(.65)	.47(.59)	1.65(.64)	.59	3	.03
Child SC reactivity	.89(.78)[a]	2.75(.65)[b]	-.17(.54)[a]	.70(.51)[a]	3.64**	3	.17
Child Startle reactivity	2.03(1.41)[b]	2.27(1.56)[b]	-3.95(1.20)[a]	5.08(1.23)[b]	8.21**	3	.27

Note: Estimated marginal means (SE)*;* Difference scores denote scores minus neutral. Different subscripts ([a,b,c]) denote significant differences between groups in post hoc pairwise comparisons. Values from the experimental phase are reported in the Table.

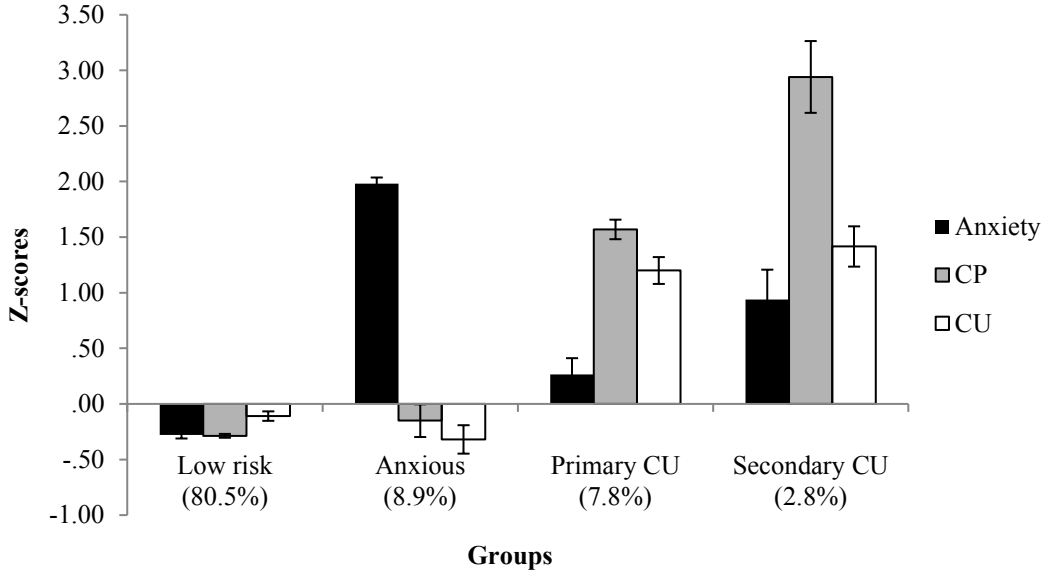

Figure 1. Z-scores and standard errors across Subgroups Identified using Latent Profile Analysis during adolescence for behavioral measures assessed initially in Study 1. CU = Callous-unemotional; CP = Conduct problems.

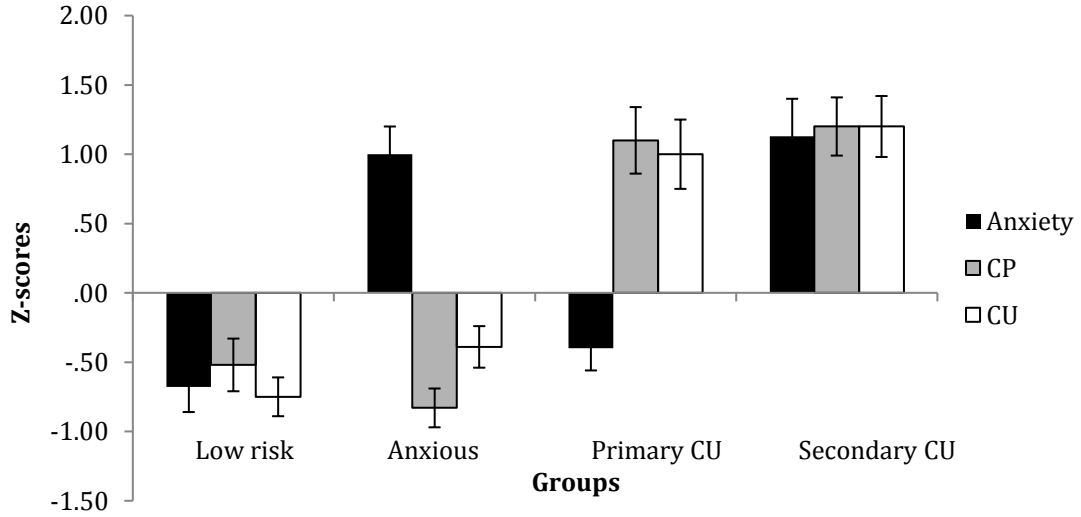

Figure 2. Z-scores and standard errors of the final groups of children participating in Study 2 using behavioral measures assessed initially. CU = Callous-unemotional; CP = Conduct problems.

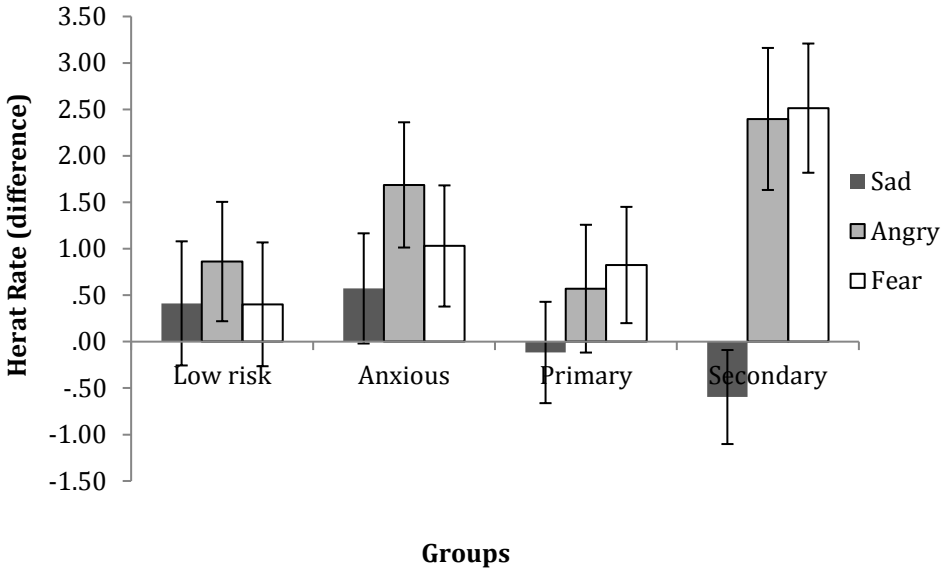

Figure 3. The significant interaction between emotions and groups predicting HR reactivity among children.

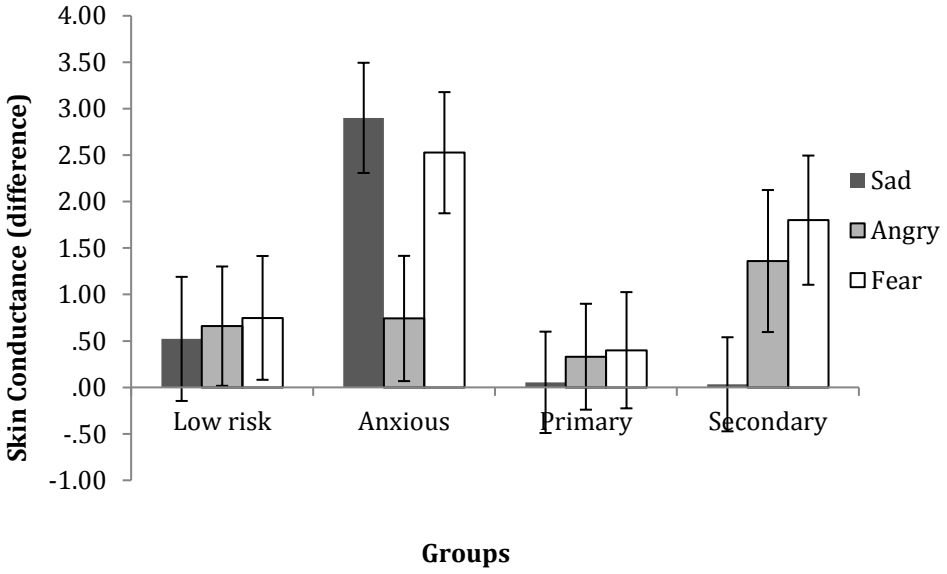

Figure 4. The significant interaction between emotions and groups predicting SC reactivity among children.